



## Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at <http://about.jstor.org/participate-jstor/individuals/early-journal-content>.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact [support@jstor.org](mailto:support@jstor.org).

# *The* Journal of Infectious Diseases

FOUNDED BY THE MEMORIAL INSTITUTE FOR INFECTIOUS DISEASES

---

*Supplement No. 3, May, 1907*

---

## ON THE USE OF METHYLENE BLUE IN TESTING SEWAGE EFFLUENTS.

EARLE B. PHELPS and C.-E. A. WINSLOW.

*(From the Sanitary Research Laboratory and Sewage Experiment Station of the Massachusetts Institute of Technology.)*

THE chief aim of sewage purification processes is to change the organic compounds of sewage into such a stable form that they will no longer tend to undergo offensive putrefactive changes. In special cases, as, for example, when effluents are discharged into tidal waters where shellfish are exposed to pollution, a high percentage removal of bacteria may also be necessary. In most instances, however, the purification plant will have done its work if it produces an effluent free from decomposable organic matter. To what extent this end is accomplished is one of the paramount questions in the control of any plant.

It is more and more clearly recognized that the intelligent operation of sewage works demands systematic analyses of the sewage and effluent at each of the principal stages in the process. The examinations which are made at different plants, however, vary widely in their precision. At the larger works complete analyses are carried out, including total organic nitrogen, suspended and dissolved, free ammonia, oxygen consumed, suspended and dissolved, nitrites, nitrates, dissolved oxygen and total solids, suspended and dissolved, with the loss on ignition. In other cases the effluents are merely examined by a rough test for putrescibility.

There is likely to be an increasing demand for more and more exact scientific control of sewage filters as sanitary standards become increasingly rigorous; rule-of-thumb methods, here as elsewhere, have probably had their best day. No conception can be formed of what goes on in a septic tank or a trickling filter or a contact bed, without knowledge of the amount and nature of the suspended matter. For example, an effluent in itself stable might easily produce in a stream a deposit of solid sludge which would eventually cause a serious nuisance. It is very helpful to the operator of a plant to know the general condition of the nitrogen and the available oxygen content at each stage of the purification process. All the analytical data noted above should therefore be obtained by the analysis of composite samples whenever possible.

The ordinary methods of analysis are, however, insufficient by themselves to furnish all the information necessary with regard to the character of a sewage effluent. Our knowledge of sewage chemistry has not yet made it possible to discriminate clearly between putrescible organic matter and those more stable "humus-like" compounds which do not undergo putrefactive decomposition. With intermittent filters, effluents are obtained which contain only a small amount of organic matter of any sort; and the purity of such effluents is easily demonstrable by oxygen consumed and nitrogen determinations. With effluents from contact and trickling filters, on the other hand, the ordinary analytical data do not offer a complete criterion of quality. An effluent may contain a considerable amount of organic nitrogen and may give a high value for oxygen consumed and yet prove of excellent quality because its organic matter is in a stable form. In such cases it becomes necessary to supplement sanitary analysis by some practical test of keeping quality. Such a test, if its technique is easy, can be made to advantage at more frequent intervals than the more elaborate chemical examinations; and in small plants where the latter are out of the question it alone may prove of considerable value.

Although the terms putrescible and non-putrescible stand for quite definite characteristics, yet the separation of effluents of various degrees of purity into these two classes depends to a considerable extent upon the test employed for the purpose. Crude sewage is

undoubtedly putrescible and a good sand effluent undoubtedly stable; but in that class of effluents in which the chemical analysis yields indefinite information, and in which the putrescibility test is most valuable, the final result is more or less dependent upon the particular technique employed.

*Historical.*—The most distinctive phenomenon associated with putrefaction is the production of offensive odors. These are due to various substances, generally gaseous, such as ammonia and the higher amido compounds, sulphides, mercaptans, and many others, all of which are associated as a rule with the anaerobic decomposition of proteid matter. It was therefore natural that the earliest tests employed to determine putrescibility should be based upon the development of the odors of these bodies shown in a sample kept in an air-tight vessel for a period of a few days. Such was the test originally devised by Scudder.<sup>1</sup> A glass stoppered bottle was filled completely with the sewage, and stored at a temperature of about 27°C. for several days. If it became dark colored and when opened gave off putrefactive odors it was considered putrescible. This so-called “smell test” was devised and used in 1895. Later, in 1899, at Manchester, the test was placed upon a more scientific footing by making initial and final determinations of the oxygen consumed from permanganate, by the “three-minute cold test;”<sup>2</sup> ferrous salts, hydrogen sulphide, and unsaturated organic bodies which are characteristic of putrefactive changes, are readily and instantly oxidized by permanganate, and hence their presence is readily detected by an increase in the oxygen-consumed figure. Modifications of the test in which the oxygen-consumed figures were determined by longer periods of contact or by boiling with the permanganate, have proved much less satisfactory, since in any such procedure a slight increase is often masked or completely nullified by a simultaneous decrease in the oxidizability of the remaining organic matter.

Later modifications of the test have been made by determining the free dissolved oxygen, and the nitrates and nitrites in the effluent before and after incubation. Such determinations show better than any other the actual course of the changes that are taking place, but the results do not always bear directly upon the question of the stability of the effluent. A marked decrease in these values does, indeed, indicate putrescible or decomposable organic matter; but if, by that decrease, the organic matter be rendered stable before the complete exhaustion of the available oxygen, then it may fairly be said that the effluent as a whole is stable. There are three possible cases. The organic matter of the effluent may be stable or it may be putrescible, i. e., capable of further anaerobic decomposition; in the latter case there may be a sufficient supply of available oxygen, free oxygen, nitrates, and nitrites present to render the organic matter stable, or the amount may be insufficient. Only in this final case is the effluent as a whole putrescible as determined by the older tests. Therefore, in interpreting determinations of oxygen dissolved, nitrates, and nitrites, it is not their diminution but their total exhaustion which indicates putrefaction.

Stoddart<sup>3</sup> made quantitative tests for hydrogen sulphide, and Dunbar and Thumm<sup>4</sup>

<sup>1</sup> *Interim Rep. Royal Commission on Sewage Disposal*, 1901, 2, Question 5988.

<sup>2</sup> FOWLER, *Sewage Works Analysis*, 1902.

<sup>3</sup> *Analyst*, 1901, 26, p. 281.

<sup>4</sup> *Beitrag zum zeitigen Stande der Abwasserreinigungsfrage*, 1902.

tested qualitatively for that gas with lead acetate paper. Both of these modifications, particularly the latter, make the test a much more severe one.

In contrast to such strict standards there has been on the other hand a tendency toward less rigid criteria. Thus Adeney<sup>1</sup> claims that an effluent discharged into a running stream containing itself an abundance of oxygen, should be diluted before testing with the waters of that stream in the proportion of their relative discharges. Clark<sup>2</sup> has used a similar method in a study of the stability of the organic matter in effluents, but as a practical test of the work of a filter it seems altogether too lenient. We have a right to demand that the effluent from a good sewage plant shall not diminish the ratio of oxidizable matter to available oxygen in the stream into which it discharges: by Adeney's test it would pass unless the entire purifying capacity of the stream would be destroyed.

An exhaustive study of the whole subject of putrescibility has recently been made by Johnson, Copeland, and Kimberly.<sup>3</sup> These investigators endeavored to find a relation between the putrescibility or stability of an effluent and its chemical analysis, particularly the oxygen consumed figures on one hand, representing oxidizable material, and the available oxygen in the form of free oxygen, nitrates, and nitrites on the other hand. The data required in establishing such a relation are:

- 1) The available oxygen in the form of free oxygen, nitrates, and nitrites, and
- 2) The "consumed oxygen," this being taken as one-fifth of the oxygen-consumed value as determined by the five-minute boiling method.

With these data the following provisional criteria of putrescibility were found to hold for the experiments in question.

When the consumed oxygen value is equal to or in excess of the dissolved oxygen figure and there are no nitrates or nitrites present the sample will putrefy.

When the consumed oxygen value is equal to or slightly less than the amount of oxygen in the effluent in the form of nitrates, nitrites, and dissolved oxygen the sample may or may not putrefy.

When the consumed oxygen value is less than the oxygen contained in the effluent in the form of nitrates and nitrites, under ordinary conditions the sample will not putrefy.

None of the tests for stability so far reviewed have proved wholly satisfactory. The "smell test" is inexact; and at the other extreme the determination of oxygen consumed, dissolved oxygen, nitrates, and nitrites, is too cumbrous a process for routine work. Furthermore, it does not accurately measure the relation of oxidizable matter to available oxygen since the oxygen consumed by permanganate bears a variable relation to the organic matter which is oxidizable under natural conditions. The "Manchester test" is somewhat more satisfactory, but this too yields abnormal results at times and only divides effluents into two rough classes without distinction as to their relative grade.

<sup>1</sup> *Rep. Royal Commission on Sewage Disposal*, 1901, 2, Question 2396.

<sup>2</sup> *An. Rep. Mass. State Board of Health*, 1902, 34, p. 373.

<sup>3</sup> *Jour. Infect. Dis.*, 1906, Supplm. 2, p. 80.

A new putrescibility test, simple in technique, and measuring accurately and delicately the relation between available oxygen and oxidizable matter, has therefore been a desideratum. Such a method appears at last to be at hand in the methylene blue test, first devised by Spitta<sup>1</sup> for the study of stream pollution and later more thoroughly worked up by Spitta and Weldert<sup>2</sup> as a test for sewage effluents. Methylene blue is a commercial dye-stuff of somewhat complex constitution, having the empirical composition  $C_{16}H_{18}N_2Cl$ . It is prepared technically by the oxidation of dimethyl-p-phenyldiamine with ferric chloride in the presence of hydrogen sulphide. The commercial product is a double chloride of zinc and the above compound. It is an extremely sensitive indicator for hydrogen sulphide and other reducing bodies, being decolorized at once in the presence of even small traces: its decolorization by bacteria has been studied by many observers, the principal of whom are cited by Spitta and Weldert.<sup>3</sup> The technique employed is extremely simple. A small portion of an aqueous solution of the dye (in our experiments 1 c.c. of a 0.1 per cent solution) is added to the effluent in a glass stoppered bottle (250 c.c. capacity in our work), and the sample is then incubated either at 20° C. or at 37° C. The blue color of the solution remains practically unchanged during the period of observation until the available oxygen contained in it is used up and putrefactive conditions arise. At this point the dye is reduced and decolorized. The time required for such decolorization is a quantitative measure of the degree of putrescibility of the sample and the retention of the color for a period of one week or more, at 20° C., or of four days at 37° C., may be taken as an indication of its stability. The criticism of Johnson, Copeland, and Kimberly<sup>4</sup> that "substances other than putrescible organic matters, such as sulphide of iron and hydrogen sulphide," discharge the color of methylene blue before the putrescible matters themselves are able to act, does not seem to us well taken, since such substances are present only when the effluent as a whole is in unstable condition.

Spitta and Weldert used both methylene blue and indonaphthol blue in their experiments, but found the latter difficult to prepare and subject to decomposition. With methylene blue, they tested 46 sew-

<sup>1</sup> *Archiv f. Hyg.*, 1903, 46, p. 113.

<sup>2</sup> *Mit. a. d. königlichen Prüfungsanstalt f. Wasserversorgung zu Berlin*, 1906, 6, p. 161.

<sup>3</sup> *Loc. cit.*

<sup>4</sup> *Loc. cit.*

age effluents of various types, mostly from contact beds and trickling beds, observing the color and odor at intervals, the total period of observation varying from one day to ten. Thirty-two samples failed to decolorize in 12 hours at 37°; and of these only one gave an odor of hydrogen sulphide, and that after two days. The other 14 samples decolorized in periods varying from an hour and a half to nine hours; all of them underwent offensive putrefaction in from 9 to 120 hours. In another series of experiments it was shown that the reduction of the methylene blue always takes place before any odor of hydrogen sulphide became noticeable. In one case the sample was decolorized after three hours and the odor was apparent only after 48 hours. In three other samples decolorization occurred after 18 hours, four days, and four days, respectively, while hydrogen sulphide could not be detected after 10 days.

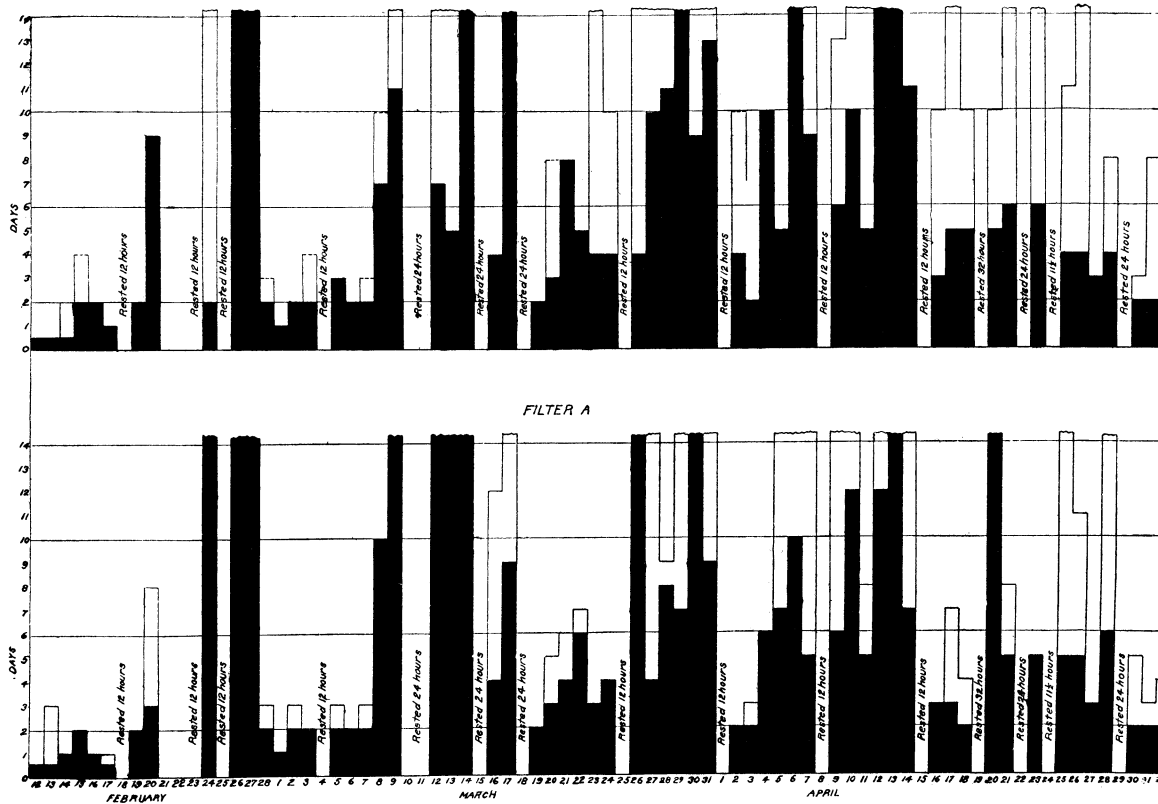
Both putrefaction and decolorization will naturally vary with the temperature at which the sample is incubated. Spitta and Weldert<sup>1</sup> give the following results, which indicate that putrefaction occurs from two to four times as fast at 37° as at the ordinary room temperature.

TABLE 1.  
EFFECT OF TEMPERATURE ON DECOLORIZATION OF METHYLENE BLUE.  
(Spitta and Weldert.)

Time of Decolorization in Hours.			
Sample	22°	28°	37°
1.....	24	6	6
2.....	3	1.5	1.5
3.....	120	96	48

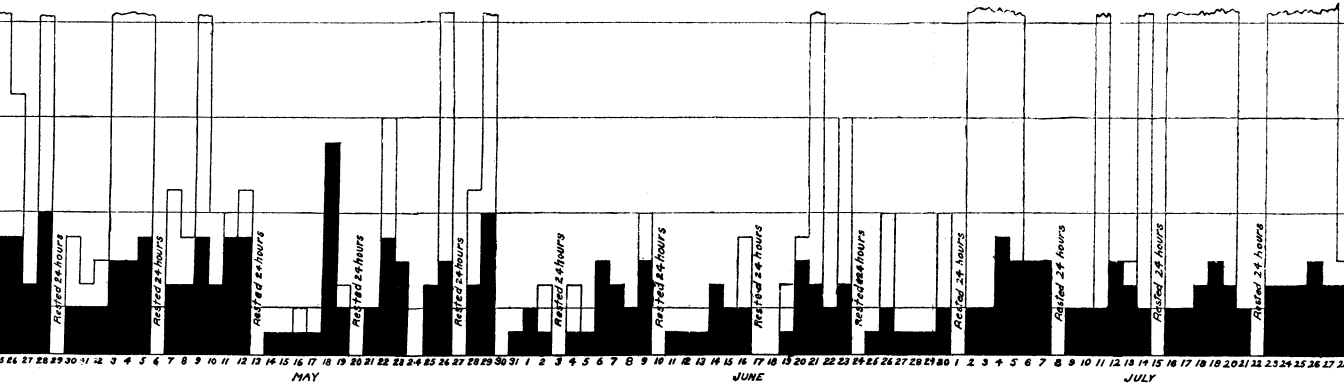
We have compared the decolorization of 64 samples of trickling filter effluent at 20° and 37° in order to obtain further light upon this point. Thirty-four of the samples were not reduced after 14 days at either temperature. In 10 cases the 20° sample retained its color for 14 days while that kept at 37° was decolorized. The period of decolorization at 20° was one day in one case (abnormality due evidently to bad sampling), from three to five days in four cases and from five to seven days in five cases. In the other 20 samples the decolorization times for both temperatures were determined to the nearest half-day and the ratios of the 20° times to the 37° times are tabulated in Table

<sup>1</sup> *Loc. cit.*

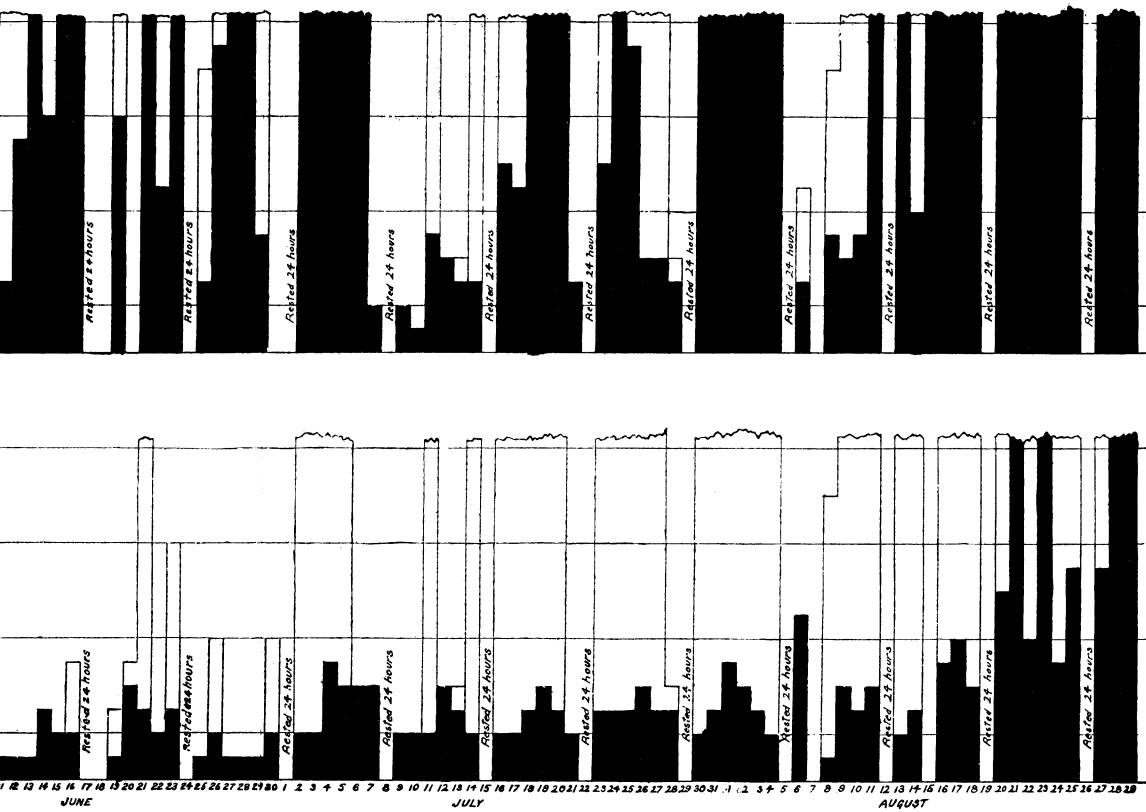


Methylene Blue Decolorization Tests. Trickling Filter Effluents. Upper series, filter B, lower series, filter A.





series, filter A, Unsettled, Solid blocks, Settled, Outlined blocks. Ordinates, Time of persistence of blue color in days at 20°. Massachusetts Institute of Technology Sewag



persistence of blue color in days at 20°. Massachusetts Institute of Technology Sewage Experiment Station.

2. The actual number of days varied from half a day to seven days at 20° and from half a day to 13 days at 37° C.

TABLE 2.  
RATIO OF TIME REQUIRED FOR DECOLORIZATION OF TRICKLING FILTER EFFLUENTS AT 20° TO THAT  
REQUIRED AT 37° C.

2.5	2.0	1.6	1.6
1.5	2.0	1.0	0.5
2.7	2.0	4.0	2.2
1.8	2.0	2.0	2.3
1.4	1.9	2.4	2.0

With the exception of two very low ratios (0.5 and 1.0), and one high ratio (4.0), all these figures fall between 1.4 and 2.7 and the average of all is exactly 2.0. It may be considered therefore that methylene blue samples will decolorize at 37° in half the time required at 20°.

It must be remembered in interpreting this table, and all others in which comparative decolorization tests are recorded, that each test must be made in a separate bottle and since the sampling of sewage is never perfect there will be variations due to the chance differences in the several bottles compared. This is particularly true in work like that later cited in Table 5 in which decolorization tests were made in one bottle and analytical data determined on another.

In general it appears from Table 2 that results obtained at 20° bear to those obtained at 37° the ratio of 2 to 1. Stable samples are stable at any temperature. In grading putrescible samples, however, it is evidently necessary to select one temperature and to use it for all comparative work. The temperature of the body has the advantage that it gives results more promptly. Any sample which does not decolorize in four days is certainly stable and a two days' limit would include almost all putrescible effluents. On the other hand, the 20° method, from the very fact that it is slower, permits a more delicate measurement of the relative differences between putrescible effluents of different grades. Decolorization here may occur from the first up to the 14th day. Furthermore, the test can be made, if necessary, at room temperature without an incubator or special apparatus; and this simplicity would be of the greatest importance in small sewage plants without a laboratory or expert supervision. Slight variations from 20° will not introduce a serious

error in the test; and diffuse daylight does not appreciably interfere with it. A series of control experiments, made in daylight, in a box at the room temperature (in summer), and in an incubator at 20°, are recorded in Table 3 and indicate that the test could be made in an ordinary room with fairly controlled temperature. Experiments made during cold weather in a small frame building heated by a stove showed marked abnormalities, on the other hand, and emphasize the fact that larger temperature variations cannot be allowed to go too far.

TABLE 3.  
EFFECT OF TEMPERATURE AND LIGHT ON METHYLENE BLUE TEST.  
Average Time of Decolorization in Days.

WEEK ENDING	EFFLUENT A			EFFLUENT B			EFFLUENT C		
	Summer Temperature		20°	Summer Temperature		20°	Summer Temperature		20°
	Daylight	Dark	Dark	Daylight	Dark	Dark	Daylight	Dark	Dark
June 27.....	1	2	2	11	11	7	2	3	3
July 2.....	3	3	3	12	12	10	3	3	4
July 9.....	2	2	2	2	3	2	2	3	3
July 16.....	2	3	2	9	12	10	13	13	13
July 23.....	2	3	4	7	8	7	14+	14+	14+
July 30.....	2	5	6	13	14+	13	12	14+	13
August 6.....	2	3	3	6	6	6	14+	13	13
August 13.....	3	5	4	13	13	12	13	13	13
August 20.....	10	5	5	14+	14+	14+	14+	14+	14+

With regard to the significance of the methylene blue test in relation to the specific chemical changes which go on in putrefaction, Spitta and Weldert have little to say beyond pointing out that decolorization takes place only after all the available oxygen has been exhausted. This they illustrate by a single experiment, cited in Table 4.

TABLE 4.  
OXYGEN CHANGES IN A TRICKLING FILTER EFFLUENT.  
(Spitta and Weldert.)

Days.....	$\frac{1}{4}$	$\frac{1}{2}$	1	2	3	4	5	10
Dissolved oxygen; c.c. per L.....	2.62	1.44	0.50	0.37	....	0.00	....	0.00
Nitrogen as nitrites and nitrites: Mg. per L	10.0	10.0	10.0	....	....	8.0	....	4.0

The methylene blue remained unchanged up to this point.

We have thought it important to study the relation between decolorization and chemical processes somewhat more fully; and the

results of the examination of five samples of trickling effluent are shown in Table 5.

TABLE 5.  
OXYGEN CHANGES IN PUTRESCIBLE TRICKLING FILTER EFFLUENTS.  
Decolorization of Methylene Blue Indicated by \*.  
(PARTS PER MILLION.)

SAMPLE	DAYS AT 37°	DISSOLVED OXYGEN	NITROGEN AS		OXYGEN CONSUMED 15 MINUTES COLD
			Nitrates	Nitrites	
1.....	Initial	8.0	3.0	1.0	5.0
	1*	0.0	0.0	0.3	5.0
	2	....	....	0.1	....
	Initial	10.0	3.0	1.0	4.0
	1	1.0	3.0	1.0	4.0
2.....	2	0.0	0.0	1.0	3.4
	3	0.0	...	0.1	4.0
	4	...	...	0.08	4.0
	5	...	...	0.03	3.8
	7	...	...	0.00	4.0
	8	...	...	...	4.8
	9	...	...	...	6.3
	Initial	8.0	3.5	0.5	6.0
	1	1.0	3.0	1.2	4.8
	2	1.2	3.0	1.4	5.7
3.....	3	0.4	3.0	1.0	5.2
	4	0.0	0.0	0.0	5.0
	7*	...	...	...	...
	Initial	10.8	4.0	0.3	4.2
	1	1.8	3.0	0.3	4.5
4..... M. B. sample lost	2	0.2	3.0	0.5	4.4
	3	0.2	3.0	0.1	3.4
	4	0.6	2.5	0.1	2.5
	5	0.0	1.0	0.1	4.3
	6	...	0.0	0.05	3.6
	7	...	0.0	0.0	2.7
	Initial	9.2	3.0	0.6	5.2
	1	1.2	2.5	0.6	5.3
5.....	2*	0.0	0.0	0.0	5.3
	3	0.0	0.0	0.0	5.7
	4	...	...	...	4.6
	5	...	...	...	3.9
	6	...	...	...	6.1
	7	...	...	...	6.9

It is evident that the free dissolved oxygen is absorbed first by the organic compounds present; then the nitrates disappear, and finally the nitrites; and the methylene blue is generally attacked just after the disappearance of the latter. Sometimes, as in sample 3, there is a considerable delay between the disappearance of nitrites and the decolorization of the methylene blue. This may probably occur when the organic matter and the available oxygen are about evenly balanced, so that after the exhaustion of the oxygen putrefactive changes are set up only somewhat slowly. Whenever the methylene blue is decolorized, it may be assumed that all oxygen as nitrates or nitrites has disappeared; and vice versa, when the blue color is retained, traces of these bodies are generally present. This

condition is illustrated in Table 6 for trickling filter effluents which did not decolorize after 14 days at 37°. It will be noticed that these effluents contained a considerable amount of organic matter as measured by oxygen consumed and that in all cases there was a

TABLE 6.  
OXYGEN AND OXIDIZABLE MATTER IN STABLE TRICKLING FILTER EFFLUENTS.  
(PARTS PER MILLION.)

INITIAL COMPOSITION				AFTER 14 DAYS AT 37°			
Dissolved Oxygen	Nitrogen as		Oxygen Consumed 15 Min. Cold	Dissolved Oxygen	Nitrogen as		Oxygen Consumed 15 Min. Cold
	Nitrites	Nitrates			Nitrites	Nitrates	
6.8	4.0	5.5	5.6	0.0	0.0	0.0	3.2
2.8	3.0	5.5	8.4	0.3	3.5	1.5	7.6
5.2*	0.0	15.0	4.4	4.0	0.1	15.0	4.4
7.2	5.0	3.0	18.8	0.1	0.4	1.5	8.9
4.4	3.0	3.0	5.2	0.0	0.1	1.5	3.5
7.2	4.0	6.0	16.8	0.1	0.5	5.0	15.8
4.6	3.0	6.0	10.4	0.0	1.5	1.0	12.2
4.4	2.5	5.5	9.6	0.0	0.0	0.0	7.0
4.4	3.0	5.5	8.4	0.2	0.8	1.5	2.0
6.6	1.0	2.0	17.4	0.0	3.0	0.1	2.6
4.2	1.5	3.0	7.2	0.0	1.0	0.0	0.6
5.7	2.5	3.0	8.4	0.0	0.0	0.0	5.2
4.3	3.0	4.0	7.0	0.6	6.0	2.0	7.7

\* Sand filter effluent.

marked reduction in oxygen content during incubation. Nitrites and nitrates remained, however, in all but three cases.

This condition may be compared with that of the putrescible effluents in Table 5 and with four other sets of analyses quoted in Table 7.

TABLE 7.  
OXYGEN AND OXIDIZABLE ORGANIC MATTER IN PUTRESCIBLE TRICKLING FILTER EFFLUENT.  
(PARTS PER MILLION.)

INITIAL COMPOSITION				AGE, HOURS AT 37°	AFTER DECOLORIZATION			
Dissolved Oxygen	Nitrogen as		Oxygen Consumed 15 Min. Cold		Dissolved Oxygen	Nitrogen as		Oxygen Consumed 15 Min. Cold
	Nitrites	Nitrates				Nitrites	Nitrates	
4.3	2.0	3.0	24.4	24	0	0	1.5	25.2
1.1	2.5	3.0	14.0	12	0	0	0.0	14.0
5.8	1.5	5.0	15.6	40	0	0	0.0	16.0
3.8	1.5	5.0	8.8	120	0	0	0.0	2.8

An interesting practical question is the relation of the methylene blue test to the original constitution of the effluent as determined by chemical analysis. If our methods were sufficiently precise we should

be able to predict from determinations of organic matter and oxygen just what the result of their interaction will be. The condition of an effluent, as indicated by the relation of "oxygen consumed" to available oxygen, corresponds, however, to its putrescibility only roughly. Johnson, Copeland, and Kimberly from their investigations made the generalizations previously quoted as to the relation between the production of odors and the analytical data; but their tables offer numerous exceptions to this general rule. Our experience with the methylene blue test has been much the same. Comparing Tables 5, 6, and 7, a general difference between the stable and putrescible samples is apparent, the former, in Table 6, having on the whole a considerably higher ratio of available oxygen to "oxygen consumed" than the latter as shown in Tables 5 and 7. There are, however, many samples which contradict this general rule, and we have been unable on any basis of calculation to find a marked and constant difference in analysis between stable and putrescible samples. This is still more forcibly brought out in Table 8 which represents the average results of determinations carried on for six months, the analytical data being determined weekly on composite samples, and the methylene blue test made six times a week. The methylene blue samples were kept for 14 days and those which retained their color for that time would probably have done so indefinitely. In calcu-

TABLE 8.  
MONTHLY AVERAGE ANALYSES OF TRICKLING FILTER EFFLUENTS AND AVERAGE DECOLORIZATION TESTS.

EFFLUENT, FILTER A.					
MONTH	OXYGEN CON- SUMED 30 MIN. BOIL	NITROGEN AS		DISSOLVED OXYGEN	METHYLENE BLUE TEST DAYS AT 20°
		Nitrites	Nitrates		
March.....	47	0.6	3.6	8.8	7
April.....	54	0.5	4.6	4.0	7
May.....	59	1.1	4.5	6.0	3.5
June.....	40	4.0	3.6	4.4	3
July.....	43	2.5	3.8	5.0	2.5
August.....	34	2.5	4.0	4.7	5

EFFLUENT, FILTER B.					
March.....	28	0.0	4.1	7.5	6.
April.....	41	0.2	5.1	3.8	8
May.....	46	1.2	4.4	5.1	4
June.....	31	2.7	3.8	4.1	10
July.....	36	2.8	5.3	4.8	10
August.....	33	3.5	4.0	5.8	11

lating averages they receive only the value of 14 days, however. The monthly averages of 10 days or more represent effluents of high stability and those of three and four days, effluents which were distinctly putrescible.

Evidently the methylene blue test does not correspond strictly to our analytical data. There are no differences in the oxygen and "oxygen consumed" values sufficient to account for the wide difference in stability between the effluents for Filter A for June and Filter B for April, for example, or between Filters A and B for August. It is evident that certain factors affect the stability of an effluent which are not revealed by the ordinary methods of sanitary chemistry. These factors must be taken into account in judging of the success of a sewage disposal plant, since the stability of the effluent is precisely the point of greatest importance.

The methylene blue test, during a year's use in the routine study of the trickling filters at the Sewage Experiment Station, has proved itself admirably adapted to the detection of slight variations in this crucial quality of stability. The general results of the test as shown in diagrammatic form on the appended folded sheet, indicate quite clearly its value. Filter A was a trickling filter 8 ft. deep and 10'  $\times$  10' in area, filled with crushed stone from 1½" to 2" in diameter and receiving crude Boston sewage at a rate of two million gallons per acre per day. Filter B was exactly similar but was dosed with the effluent from a septic tank of seven hours' capacity. The block diagrams refer in each case to the effluents as they flowed from the filters; and the outline diagram to the same effluents settled for two hours in tanks of a modified Dortmund pattern. It will be noticed that the two filters gave similar results during the first winter, the unsettled effluents being generally putrescible and the settled effluents generally stable. In April both fell off somewhat, but in May the filter taking septic sewage improved in operation and all through the summer gave effluents which even without sedimentation were generally stable. Filter A, receiving crude sewage, continued to deteriorate. Through June even its settled effluent usually putrefied and in July and August its effluents were stable only after sedimentation. Here were radical differences in the work of two filters, so radical that one might be considered a failure and the other a success. Yet



the analytical data for these same filters, as given in Table 8, offered no adequate hint of the distinction.

We believe, therefore, that some special test for stability is urgently needed in all sewage analysis; and the methylene blue test seems to us to have certain special advantages, and to deserve wider attention than it has so far received. It directly measures the quality of most importance in a sewage effluent, its freedom from the tendency to putrefactive change. It registers this with a greater delicacy than any method with which we are acquainted. The range between an effluent which decolorizes in 24 hours and one which retains its color for two weeks makes it possible to distinguish a dozen grades of effluents between the highly putrescible and the certainly stable one. The effect of the Sunday resting-period in decreasing the stability of the effluents shown on the diagram is a striking instance of the way in which this test reveals slight derangements of a delicately organized filter. If results are desired promptly the test may be made at  $37^{\circ}$  and will then distinguish between a putrescible and a stable effluent in two days. It is so simple in technique as to be peculiarly adapted to sewage works where neither a laboratory nor an expert chemist are available.